Published in final edited form as:

Invest Ophthalmol Vis Sci. 2009 January ; 50(1): 37–46. doi:10.1167/iovs.08-2102.

Temporal Properties of Compensation for Positive and Negative Spectacle Lenses in Chicks

Xiaoying Zhu, M.D. and Josh Wallman, Ph.D.

Department of Biology, City College of the City University of New York

Abstract

Purpose—Chicks' eyes rapidly compensate for defocus imposed by spectacle lenses by changing their rate of elongation and their choroidal thickness. We hypothesize that defocus causes internal emmetropization signals to rise and become saturated during episodes of lens-wear and to decline between episodes. We measured the time-constants of these signals indirectly by measuring the magnitude of lens-compensation in refractive error and ocular dimensions as a function of the duration of episodes and the interval between the episodes.

Methods—First, to study how quickly the signals rose, we gave chicks episodes of lens-wear of various durations (darkness otherwise) and estimated the duration required to cause a half-maximal effect (rise-time). Second, to study how quickly the signals declined, we gave episodes of lens-wear with various dark intervals in between and estimated the interval required to reduce the maximal effect by half (fall-time).

Results—The rise-times for the rate of ocular elongation and choroidal thickness were approximately 3 min for positive and negative lenses. The fall-times had a broad range of time-courses: Positive lenses caused an enduring inhibition of ocular elongation with a fall-time of 24 hr. In contrast, negative lenses caused a transient stimulation of ocular elongation with a fall-time of 0.4 hr.

Conclusions—The effects of episodes of defocus rise rapidly with episode duration to an asymptote and decline between episodes with a time-course that depends strongly on the sign of defocus and the ocular component. We suspect that the complex etiology of human myopia may reflect these temporal properties.

Keywords

emmetropization; myopia; hyperopia; ocular length; choroid

During early post-natal life, ocular growth is modulated by the visual input from the retina, resulting in the correction of refractive errors (reviewed by refs. 1–4). Eyes use two compensatory mechanisms to reduce defocus: When the eye wears a positive lens, which would put the images of distant objects in front of the photoreceptors (myopic defocus), it slows its rate of elongation and thickens the choroid, pushing the retina forward toward the image plane. When the eye wears a negative lens, which would put the images of distant objects behind the photoreceptors (hyperopic defocus), it accelerates its rate of elongation and thins the choroid, pulling the retina back toward the image plane. Compensation by changing the eye size has been found in fish⁵, chicks^{6,7}, guinea pigs⁸, tree shrews^{9,10}, cats¹¹, marmosets¹², and rhesus

Corresponding author: Xiaoying Zhu, Biology Department, The City College of New York, 138th St. and Convent Ave., New York, NY, 10031, Telephone: 212-650-8459, Fax : 212-650-8451, E-mail: xiaoying@sci.ccny.cuny.edu.

Commercial relationships policy: N.

monkeys¹³. Compensation by changing choroidal thickness has been shown in chicks¹⁴, tree shrews⁹, guinea pigs¹⁵, marmosets¹⁶, and macaque monkeys¹⁷.

In normal life, of course, the retina does not exclusively experience continuous myopic or hyperopic defocus. Rather, most parts of the retina experience frequent episodes of myopic and hyperopic defocus depending on the spatial layout of the environment, the distance of the objects viewed, and the eye's refraction and its accommodative state. How does the retina sum together these episodes of defocus over time to determine the direction of the eye's growth toward emmetropia?

The simplest model of integration would be that the defocus is summed linearly, i.e., the resulting compensation is proportional to the average amount of defocus that the retina experiences summed over a period of time (Fig. 1A). If the eye were experiencing myopic defocus, for example, the emmetropization signal favoring hyperopic growth would keep rising as long as the defocus is present. When there is no defocus (e.g., in darkness), the signal would remain stable. When the sign of defocus reverses, the signal would switch direction.

Despite of its appealing simplicity, there is evidence against this linear model. When chicks had the same total amount of lens-wear (28 min) each day given in various numbers of episodes with darkness in between¹⁸: (1) Many episodes of lens-wear (14 episodes, 2 min each) were more effective than one single episode (of 28 min) in causing compensation for either positive or negative lenses, in agreement with findings that several episodes of normal vision each day were more effective than a single episode of the same total duration in reducing form-deprivation myopia in chicks¹⁹. These results imply either that the hypothesized internal emmetropization signal reaches saturation within an episode or that it declines between episodes. (2) Very brief episodes were ineffective at causing compensation, even if repeated very often (2-sec episodes given every 2 min for a total duration of 28 min), suggesting that the signal must accumulate to threshold within each episode before downstream effects are initiated.

In light of the above evidence, we hypothesize that episodes of myopic and hyperopic defocus are integrated in a nonlinear fashion, as proposed by Flitcroft²⁰: Lens-wear causes one or several internal emmetropization signals to rise and eventually become saturated during episodes of lens-wear and decline slowly between episodes (Fig. 1B).

As a start to exploring this hypothesis, we studied the time-constants of the signals regulating compensation for positive and negative lenses. Because we do not know the nature of these signals, we cannot measure them directly, but can measure their effects on the rate of ocular elongation and choroidal thickness. Because a single brief episode of lens-wear would not generally have a measurable effect, we estimated the rise- and fall-times of these inferred signals by measuring the eye before and after 3 days of many episodes of viewing through positive or negative lenses: (a) We estimated how quickly the hypothesized signals rise during each episode by giving frequent episodes of lens-wear of a range of durations, reasoning that the ocular responses will increase with increasing duration up to an asymptote. (b) We estimated how quickly the signals decay between episodes by giving long (saturating) episodes of lens-wear with a range of intervals between the episodes, reasoning that the ocular responses will decline with increasing intervals.

We find that the signals for the effects of both positive and negative lenses on both the rate of ocular elongation and choroidal thickness rise at a similar rate (within minutes), but they decline at slower, very different rates, with the signal regulating the rate of ocular elongation declining the slowest in the case of positive lens-wear, and the fastest in the case of negative lens-wear. Some of these results have been presented in an abstract (Zhu X, *et al.* *IOVS* 2004;42:ARVO E-Abstract 4285).

MATERIALS AND METHODS

Animals

White Leghorn chicks were obtained from either Cornell University (Cornell K-strain; Ithaca, NY) or Truslow Farms (Hyline-W98-strain; Chestertown, MD; all of group 14 and part of groups 8, 11 and 13); because we did not find any statistically significant differences between the strains in the degree of compensation, results were pooled for analysis. Before the experiments started, chicks were reared in heated brooders under fluorescent lighting (lights off from 10 pm to 8 am). During experiments chicks were housed in a heated, sound-attenuated chamber (76 × 61 cm), with the lights off from 8–8:30 pm (depending on the experimental group) to 8 am. The timing of illumination for the experimental groups is shown in Table 1. Birds were kept in darkness between episodes. Food and water were provided *ad libitum*. Care and use of animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experiments started when the chicks were 1 week old and lasted for 3 days.

Lenses

PMMA plastic lenses (with a back optic–radius of 7 mm) or glass lenses (not conspicuously curved) of +7 and −7 D lenses were used. The lenses (diameter = 12 mm) were glued between rigid plastic rings and Velcro rings attached to mating Velcro rings glued to the feathers around the chicks' eyes. Lenses were cleaned at least twice a day. In both experiments, chicks had monocular lens-wear, leaving the other eye uncovered.

Measurements

Measurements of refractive error and ocular dimensions were conducted on chicks anaesthetized with 1.5% halothane. Birds were measured at the same time of day before and after the 3 days of lens-wear. Refractive error was measured using a modified Hartinger refractometer.²¹ A-scan ultrasonography was used to measure internal ocular dimensions.²² Ocular length was measured from anterior cornea to posterior sclera (the sum of anterior chamber depth, lens thickness, vitreous chamber depth, and the thickness of retina, choroid and sclera), using appropriate sound velocities for each ocular component.²¹ This differs from conventional axial length measurement, from cornea to retina, which is affected by changes in choroidal thickness and therefore confounds ocular length with choroidal thickness, which, as we will show, are controlled separately.

Protocols

Treatments and lens-wearing paradigms for experiments 1 and 2 are listed in Table 1.

Experiment 1. Estimation of rise-time—To characterize how fast the hypothesized internal emmetropization signals rise, we estimated the episode-duration that produced lens-compensation half as effective as continuous lens-wear (called the rise-time), by giving the birds repeated episodes of light with lens-wear for various durations, with darkness in between (see Fig. 2A for the schematic and Table 1 for details). Chicks wearing −7 D lenses were given episodes of various durations every 10 min^{*}; those wearing +7 D lenses were given episodes every hr, except for two groups of birds (groups 1 and 5) which were given episodes every 10 min to assess the influence of the frequency of episodes of a given duration. Two control groups of chicks wore positive (group 8) or negative (group 13) lenses continuously under a normal 14:10 light:dark cycle.

*In a preliminary experiment, when chicks wearing negative lenses were in darkness except for 10 sec every hr over 3 days, choroidal thinning ($-139 \pm 24 \mu\text{m}$, Mean $\pm$ SEM) and a myopic shift (-2.9 ± 0.6 D) occurred in the fellow eye, which limited how much additional thinning could be produced by negative lenses. To prevent this dark-induced thinning, we gave −7 D lens-wear every 10 min.

Experiment 2. Estimation of fall-time—To characterize how fast the hypothesized internal emmetropization signals fall, we studied the interval in the dark between episodes that produced lens-compensation half as effective as continuous lens-wear (called the fall-time), by giving long (30 min) episodes of light with +7 or -7 D lens-wear with various dark intervals in between (see Fig. 2B for the schematic and Table 1 for details). Since Experiment 1 showed that the rise-times for both positive and negative lenses were 5 min or less, we gave chicks 30-min episodes of viewing through lenses to ensure a saturating effect of each episode, with various dark intervals from 0.5 hr (one episode every hr) to 47.5 hr (one episode every 2 days). When only one episode of lens-wear was given every day, it took place at the same time each day (between 12 and 2 PM). When two daily episodes were given, they took place from 8:00 to 8:30 AM and 8:00 to 8:30 PM, resulting in a dark interval of 11.5 hr. When more frequent episodes were given, the first episode was at 8 AM the last at 8 PM.

Statistics and Calculations

Changes in experimental eyes and fellow eyes over the 3-day-long experiments are summarized in Table 1. Because we were specifically interested in the changes during the course of the treatment, we used Relative Change (the change in the experimental eye over the course of the experiment minus the change in the fellow eye) to represent the net effect of the lens-wear. The Relative Changes that were significantly different from 0 by paired, 2-tailed Student *t*-tests are shown in Table 1. Relative Change is plotted against either the duration of the lens-wearing episodes for rise-time in Exp. 1 (Fig. 3) or the dark interval between lens-wearing episodes for fall-time in Exp. 2 (Fig. 4) both on logarithmic scales, so different time points are nearly evenly separated. Analysis of Variance (ANOVA) with LSD *post-hoc* tests were used to compare the relative changes among various episode durations (for Exp. 1) or dark intervals between episodes (for Exp. 2).

Data of the individual birds of each experiment were fitted (using Igor Pro version 5.02) with sigmoidal curve of the form $y = y_0 + \Delta y / (1 + \exp^{(x_0 - x)/z})$, with x being the logarithm of the episode duration or interval. The coefficient y_0 is the y value at small x , $(y_0 + \Delta y)$ is the y value at large x , x_0 is the log of the x value at which y is midway between the low and high y -asymptotes (i.e., the rise- or fall-times), and z is the rate of rise or fall. The 95% confidence intervals (CI) for x_0 were also calculated. In some cases, the unconstrained curve-fitting yielded implausible confidence intervals. Therefore, in all cases, we did 3 successive fits to yield x_0 , the primary parameter of interest: First, we limited the ranges of y_0 and Δy and let the algorithm fit these 2 parameters to yield the fitted values for y_0 and Δy ; second, we fixed y_0 and Δy at their fitted values and let the algorithm fit the optimal value for z ; and third, we fixed y_0 , Δy , and z at their fitted values and let the algorithm obtain the value for x_0 and its 95% confidence interval. Because x_0 was calculated as a logarithm, the 95% CI was asymmetric when converted into minutes or hours.

RESULTS

The findings presented here support our hypothesis that lens-wear causes rapidly saturating effects, which decay during subsequent darkness. When chicks viewed the world through defocusing lenses for episodes lasting 1 min or less, no effect was seen, but with episodes lasting a few minutes the effects rose rapidly to being as great as if the chicks had worn the lenses continuously under normal illumination. When long episodes of viewing through lenses were followed by intervals of darkness, the effects differed dramatically in their persistence: The inhibition of ocular elongation caused by positive lens-wear persisted for many hours, whereas the stimulation of ocular elongation caused by negative lens-wear was gone after only half an hour of darkness. For simplicity, we will present the main results in terms of the changes over the experiment in the lens-wearing eye minus the change in the fellow eye, termed Relative

Change. The changes in ocular length, choroidal thickness, vitreous chamber depth, and refractive error for lens-wearing and fellow eyes are summarized in Table 1.

Experiment 1. Estimation of rise-time

As expected, compensation by changes in ocular elongation (Fig. 3A) and choroidal thickness (Fig. 3B) was not simply a linear function of the lens-wearing duration, but rather a sigmoidal function. The rise-times were very similar for both ocular elongation and choroidal thickness when either positive or negative lenses were worn, ranging from 1.3 to 4.4 min.

Ocular Length—The degree to which the rate of ocular elongation was affected by lens-wear was determined by the episode duration: When viewing through +7 D lenses was given 10 sec every 10 min (group 1, Table 1), little ocular compensation was found (Fig. 3A); vision for 30 sec or 1 min every hr (groups 2 and 3) resulted in small but consistent ocular inhibition, and further increasing the duration to 2 min or more every hr caused the maximal inhibition of ocular elongation, like that with continuous positive lens-wear (relative change: $-183\ \mu\text{m}$ vs. $-195\ \mu\text{m}$, groups 4 vs. 8). Durations of 2 min or longer showed a significantly greater inhibition than durations of 1 min or shorter ($p < 0.05$, ANOVA with LSD *post-hoc* test, see Fig. 3A). The rise-time for ocular inhibition caused by wearing positive lenses was 1.3 min (95% CI: 0.8–1.9 min), whether we used the data from the hourly episodes (group 4), or the every-10-min episodes (group 5), suggesting that the lens-wearing frequency did not have a big impact on the rise-time.

Viewing through $-7\ \text{D}$ lenses for 10 sec or 1 min every 10 min (groups 9 and 10) caused 30% and 45% of the maximal increase in the rate of ocular elongation seen in continuous negative lens-wear (group 13), respectively; further increasing the lens-wearing duration to 5 min caused the maximal increase in the rate of ocular elongation. The two longest durations (5 and 10 min) had a significantly greater effect than did 10 sec (relative change, 5 or 10 min vs. 10 sec, $p < 0.05$, ANOVA with LSD *post hoc* test). The rise-time for ocular elongation caused by negative lens-wear was 1.6 min (95% CI: 0.5–5.2 min).*

Choroidal Thickness—Similar to their effect on inhibition of ocular elongation, short durations of viewing through positive lenses caused little choroidal expansion (10 sec to 2 min, groups 1 to 4), but a longer duration (5 min, group 6) caused a robust choroidal expansion (relative change, $58\ \mu\text{m}$, Table 1 and Fig. 3B). When the duration was further increased to 10 min, choroidal thickening reached the maximum (group 7, $132\ \mu\text{m}$, relative change), significantly more than found when durations were 2 min or shorter ($p < 0.01$, ANOVA with LSD *post-hoc* test, see Fig. 3B). The rise-time for choroidal thickening was 4.4 min (95% CI: 2.3–8.4 min) using the data from hourly episodes (group 4), or 5.0 min using the data from the every-10-min episodes (group 5).

For negative lens-wear, although the shortest duration did not cause any choroidal thinning (10 sec/10 min, group 9), longer durations (1 to 5 min, groups 10 to 12) caused from 25% to 73% of maximal choroidal thinning (group 13). The rise-time of choroidal thinning with negative lens-wear was 2.9 min (95% CI: 1.1–8.2 min).

In summary, the signals modulating both the rate of ocular elongation and choroidal thickness for both positive and negative lens-wear rose at a similar speed, with the rise-time ranging from 1.3 to 4.4 min. No statistical difference was found.

*Curiously, viewing through $-7\ \text{D}$ lenses for 2 min every 10 min (group 11) had no effect on the rate of ocular elongation in 2 separate experiments with a total of 15 birds. The negative lens-wearing eyes elongated less than the fellow control eyes in 10 out of 15 birds (Mean $\pm$ SEM: $-7 \pm 22\ \mu\text{m}$, relative change). Including this group in the curve-fitting changed the rise-time to 3.7 min (95% CI: 2.5–5.4 min, see the dashed line in Fig. 3A).

The rise-times for vitreous chamber depth and refractive error were both less than 4 min for both positive and negative lens-wear (data not shown). The sum of the changes in vitreous chamber depth and in choroidal thickness well correlated with the changes in ocular length (slope = 0.92, $r^2 = 89.7\%$ for relative change). No difference was found between paired eyes for either anterior chamber depth or lens thickness.

Experiment 2. Estimation of fall-time

Similar to the rise-times found in Experiment 1, the decay of the signals controlling the rate of ocular elongation (Fig. 4A) and choroidal thickness (Fig. 4B) was a sigmoidal function of the dark interval between lens-wearing episodes. Unlike the rise-times, the signals declined not only more slowly, but also with dramatically different patterns depending on the sign of defocus and the ocular component. The fall-times ranged from 0.4 hr to 24.4 hr. Episodes of positive lens-wear had a more enduring effect on both the rate of ocular elongation (fall-time 24.4 hr) and choroidal thickness (6.7 hr) than did episodes of negative lens-wear (fall-times for the rate of ocular growth and choroidal thickness: 0.4 and 3.2 hr, respectively).

Ocular Length—One of the most striking findings was that the signal regulating the rate of ocular elongation with positive lenses was very enduring: viewing through positive lenses for 30 min with dark intervals from 1.5 to 11.5 hr all caused more than 90% of the maximal inhibition of ocular elongation found with birds wearing positive lenses continuously (groups 14 to 17, Fig. 4A). When the dark interval was increased to 23.5 hr, i.e., one episode per day, ocular inhibition was still 70% of the maximum (group 18), significantly less than the inhibition with the dark intervals 3.5 hr or shorter ($p < 0.05$, ANOVA with LSD *post-hoc* test, see Fig. 4A). Even viewing through positive lenses once every 2 days (dark interval 47.5 hr, group 19) still caused about one third of the maximal inhibition, significantly less than the inhibition with most of the dark intervals of 23.5 hr or shorter ($p < 0.05$, ANOVA with LSD *post-hoc* test, see Fig. 4A). The fall-time for the rate of ocular elongation with positive lenses was 24.4 hr (95% CI: 20–30 hr).

In contrast to the enduring signal found in positive lens-wear, the signal for negative lens-wear was transient: dark intervals of only 0.5 hr significantly reduced the rate of ocular elongation by two thirds, compared to continuous lens-wear (for dark interval, 0.5 hr vs. continuous lens-wear, groups 20 vs. 13, $p < 0.01$, ANOVA with LSD *post-hoc* test); longer dark intervals eliminated the effect of the lens-wear (all groups were significantly less than that with continuous lens-wear or with 0.5-hr dark interval). The fall-time for the rate of ocular elongation with negative lenses was 0.4 hr (95% CI: 0.2–0.9 hr). This fall-time was so much shorter than that for positive lens-wear that even their 99% confidence intervals do not overlap.

Choroidal Thickness—Viewing through positive lenses every 2 to 6 hr (dark intervals 1.5 to 5.5 hr, groups 14 to 16) caused about 80% of maximal choroidal expansion, but with dark intervals 11.5 hr or longer (groups 17 to 19), no choroidal expansion was evident (relative change, continuous lens-wear, 3.5 or 5.5 hr vs. 11.5 or 27.5 hr, $p < 0.05$, ANOVA with LSD *post-hoc* test). The fall-time for choroidal thickening was 6.7 hr (95% CI: 5.0–8.8 hr).

Viewing through negative lenses every 4 hr or less often (dark intervals 3.5 hr or longer, groups 22–24) reduced choroidal thinning, but, not significantly different from viewing through negative lenses continuously. The fall-time for choroidal thinning was 3.2 hr (95% CI: 1.8–5.4 hr), a value not significantly different from the fall-time for positive lens-wear.

Given that the fall-time was greater for positive than for negative lens-wear for both ocular length and choroidal thickness (Fig. 4), it is not surprising that the fall-times for vitreous chamber depth followed this same pattern, with the fall-time of 17.2 hr (95% CI: 14.4–20.6 hr) for positive lens-wear, significantly longer than that for negative lens-wear (2.2 hr, 95%

CI: 1.7–2.9 hr). On the other hand, for refractive error, the fall-time for negative lens-wear (17.4 hr, 95% CI: 9.3–32.5 hr) was longer than the fall-time for positive lens-wear (5.5 hr, 95% CI: 5.3–5.8 hr), although we are inclined to doubt this finding because the confidence interval for the negative-lens wear was much larger than for any of our other measurements.

Again, the sum of the changes in vitreous chamber depth and in choroidal thickness well correlated with the changes in ocular length (slope = 0.93, $r^2 = 87.4\%$ for relative change). No difference was found between paired eyes for either anterior chamber depth or lens thickness.

DISCUSSION

Our results are consistent with the existence of emmetropization signals that increase to saturation during episodes of defocus and then decline between episodes. Furthermore, there seem to be separate signals for the ocular elongation and choroidal components of lens-compensation, as well as differences between positive and negative lenses, suggestive of separate signals for myopic and hyperopic defocus. The signals regulating the rate of ocular elongation and choroidal thickness with both positive and negative lens-wear rise at a similar rate (1 to 4 min required to increase it by 50%), and fall at drastically different rates: The signal for inhibition of ocular elongation by positive lens-wear is the most enduring (a 24.4-hr dark interval is required for a 50% decrease), and the signal for acceleration of ocular elongation by negative lens-wear is the most transient (only a 0.4-hr dark interval is required for a 50% decrease); the signals controlling choroidal thickness are intermediate.

Relation to previous studies

The results presented here generally confirm the results from a previous study in which only a few durations and intervals were tested (plotted as “a” and “b” in Fig. 3 & Fig. 4).¹⁸ Specifically, the fall-times for positive lenses were nearly identical with respect to both choroidal thickness and ocular elongation, although our results yield both rise-times for positive lenses and fall-times for negative lenses that are somewhat longer for choroidal thickness and slightly shorter for ocular elongation than those of Winawer *et al.* Our results also confirm those of another study²³, in which chicks were given a single 10-min episode of positive lens-wear, which produced robust choroidal expansion, consistent with the 4.4-min rise-time reported here. This choroidal expansion lasted for 6 hr, consistent with the 6.7-hr fall-time reported here.

If we compare our results to those of a previous study in which chicks had normal visual experience in between the episodes of positive lens-wear (triangles in Fig. 3 and Fig. 4),²⁴ we find that the rise-time for choroidal expansion was identical, but the fall-time seemed a bit shorter in the birds with normal vision. Unfortunately, the changes in ocular length in that study were so small (because of the preponderance of normal vision) that one cannot infer the rise- and fall-time from the two points of data available in that study.

Our finding that the effect of negative lenses is very transient, whereas that of positive lenses is surprisingly enduring might explain the temporal asymmetries between wearing positive and negative lenses observed by others. First, only 1–4 hr of unrestricted vision each day completely cancelled out the effect of wearing negative lenses or diffusers the remainder of the day in chicks^{25,26}, tree shrews²⁷, and monkeys^{28,29} (see ref. 28 for a comparison of the effect of unrestricted vision on myopic development across species). Second, wearing positive lenses for just 1 hr each day, with unrestricted vision the rest of the day, still caused hyperopic development.^{24,26}

Furthermore, although brief periods of wearing either positive or negative lenses produced approximately equal amounts of compensation, alternating positive and negative lens-wear or

alternating myopic and hyperopic defocus favored the compensation for positive lenses¹⁸ or myopic defocus³⁰, even if the duration of positive lens-wear (four 2-min episodes in chicks²⁴) was much shorter than that of negative lens-wear (the remainder of the day). A similar, but less consistent, effect was caused by an episode of 45 min of positive lens-wear in tree shrews.³¹ And when hyperopic and myopic defocus is alternated rapidly by an optical trick in chicks, the balance between the efficacy of the myopic and hyperopic defocus depends strongly on the frequency of alternation: At low frequencies, the myopic defocus dominates; at high frequencies (2 Hz), the myopic and hyperopic defocus is equally effective.³⁰ Our findings that the inferred emmetropization signals of both components of compensation for positive lens-wear have longer fall-times than those for negative lens-wear (in case of ocular length, 50 times as long) might help explain why positive lens-wear can so potently reverse the effects of negative lens-wear.

Separate signals regulating the rate of ocular elongation and choroidal thickness

Our findings add to the evidence that choroidal expansion is controlled separately from ocular elongation, in that for negative lenses the fall-time for choroidal thinning is eight times that of ocular elongation, and for positive lenses the fall-time for choroidal thickening is one quarter that of the inhibition of ocular elongation. Furthermore, there are conditions under which we find robust compensation for one of the components and no compensation for the other. For example, with 30-min episodes, twice per day (dark interval 11.5 hr, group 17), only inhibition of ocular elongation (by 204 μm), but not choroidal thickening, occurred in the case of positive lens-wear, whereas the opposite was found in the case of negative lens-wear (a substantial choroidal thinning of 54 μm , without ocular elongation, group 23). Therefore, it appears that ocular and choroidal compensation are driven by different signals.

The dissociation of ocular and choroidal compensation has been reported previously: (1) brief periods of strobe light at dawn and dusk in eyes wearing translucent diffusers^{32,33} and brief periods of light during the night in eyes wearing negative lenses³² reduce the rate of ocular elongation with little effect on choroidal thinning, (2) twice daily 30-min episodes of spectacle lens-wear (with darkness in between) produces an inhibition of ocular elongation without choroidal thickening in the case of positive lens-wear, and choroidal thinning without ocular elongation in the case of negative lens-wear¹⁸, similar to our findings, (3) interrupting all-day-long binocular negative lens-wear by brief periods of positive lens-wear on one eye and plano lens-wear on the other eye produces greater inhibition of ocular elongation in the positive-lens-wearing eye without any choroidal thickening²⁴, and (4) wearing a weak diffuser on top of a positive lens reduces choroidal expansion and enhances the inhibition of ocular elongation, compared with the fellow eye wearing a positive lens alone³⁴.

Nickla has shown that the inhibition of ocular elongation in several different visual conditions is always accompanied by a transient choroidal thickening.³⁵ Our present findings that viewing through positive lenses separated by long intervals of darkness can cause ocular inhibition without choroidal thickening are not necessarily in conflict with these results, because transient choroidal expansions might have occurred with no cumulative effects.

Possible mechanisms underlying the range of time-courses

It is striking that only minutes of vision are required to produce near-maximal responses to either sign of lens, and that the responses last at least ten times as long in darkness. We speculate that the rise-time is set by retinal processes, possibly with the same processes affecting changes in both ocular elongation and choroidal thickness. Once the changes are initiated, however, they may set into motion processes in other tissue layers whose endurance is determined by different mechanisms for each response. Thus, we can imagine that the stimulation of ocular elongation may be under the control of growth factors, such as TGF β ³⁶, the biological

availability of which could be rapidly modulated, yielding short fall-times, whereas initiating the transcription of the genes for the enzymes that produce a growth-inhibiting molecule like retinoic acid³⁷ or glucagon³⁸ might inhibit ocular elongation either directly in the sclera or indirectly via other tissues long after the initiating molecule was gone. If the initiating molecule also initiated choroidal changes, this could result in one molecule causing effects with two different time courses. Furthermore, the expansion of the choroid might involve active transport of osmotically active molecules into the lacunae of the choroid, whereas the thinning may result from either control of the lymphatic valves from lacunae to veins or by passive transport of water and ions out of the lacunae, which might have a very different time-course.

Effects of darkness

Because the chicks in this study were kept in darkness for much of the time, we need to discuss whether the darkness vitiates our results. To be sure, the ideal way to assess the temporal characteristics of the lens-wear would be to keep the birds between episodes in a neutral condition that had no influence on the ocular parameters. Unfortunately, darkness is the condition closest to neutral that we are aware of. In the case of positive lenses, it is possible to see effects of brief episodes of lens-wear when these episodes are separated by periods of normal vision²⁴, although the effects are much smaller than those shown here. As mentioned in the Results, from that study we could estimate the effect on the choroid rise-time, which was the same as measured here, and on the choroid fall-time, which may be a few hours shorter than measured here. For negative lenses, however, even brief periods of normal vision would eradicate the effect of the episodes of lens-wear. Thus, we can estimate the effect of the darkness on the fellow eyes, but if darkness interacted with the time-course of the response to the lenses, it would not be discernable in our results.

In spite of this obstacle, we estimated the effect of darkness, *per se*, dissociated from the effect of the lenses, by comparing the fellow eyes of the different groups of Experiment 2 (Fig. 5A). (Changes in the fellow eyes from Experiment 1 are comparable to those from Experiment 2.) Clearly, the choroids are progressively thinned in longer periods of darkness. This thinning might limit the extent of further choroidal thinning to negative lenses, but would be less of a problem for eyes wearing positive lenses, which cause the choroids to expand. We are encouraged by the fact that in the birds wearing negative lenses with the longest dark interval (47.5 hr, group 25), the fellow eyes only thinned by 23 μm . Nonetheless, this dark-induced thinning may be responsible for the fact that, even after subtracting the changes in the two eyes, the choroid thinning does not fall to zero with long intervals between episodes (Fig. 4B). On the other hand, long periods of darkness only cause a small increase in the rate of ocular elongation (Fig. 5B).

Effects of other sources of variation

In addition to responding to the lenses we imposed, the eyes may have been adjusting their choroidal thickness and ocular length as part of their natural emmetropization process. Thus, eyes that had been myopic or hyperopic prior to our experiment may have previously thickened or thinned their choroids in response, and may be readjusting their choroids back to normal thickness (Fig. 6). Not surprisingly, this effect is particularly robust when the birds were kept in the darkness between episodes (slope = -0.8 , filled symbols in Fig. 6), and significantly weaker when the birds were given light between episodes and thus had stronger visual influences on choroidal thickness (slope = -0.6 , open symbols, data from ref. 24), the slopes of these two groups are significantly different). A similar normalizing was seen with respect to refractive error (data not shown), although the starting choroidal thickness and refractive error were not correlated ($r^2 = 6.9\%$).

Furthermore, although it is well established that, although the two eyes of chicks respond substantially independently to lens-wear, there is some degree of yoking of the two eyes, such that the choroidal thickness and rate of ocular elongation of the fellow eye changes in the same direction, but to a much smaller degree (10–30 percent, in our data), as does the lens-wearing eye (Fig. 5C and 5D).³⁹

As an estimate of the relative contribution of these two factors, plus that of darkness, on the choroidal thickness of the fellow eyes, an ANOVA indicated that the hours of darkness accounts for much less of the variance ($F=15$) than does the initial thickness of the choroids ($F=111$), but more than the changes in choroidal thickness of the lens-wearing eyes ($F=5.1$).

In the course of this study, we discovered a new form of yoking between the eyes: Although there is a positive correlation between the fellow eye and the lens-wearing eye, the mean of the fellow eye changes in the opposite direction as the mean of the lens-wearing eye. Thus, although the choroids are, of course, thicker in eyes wearing positive lenses than those wearing negative lenses, the fellow eyes of those wearing negative lenses are significantly thicker than the fellow eyes of those wearing positive lenses ($p < 0.05$, paired 1-tailed *Student t*-test, Fig. 5A and 5C). A colleague has discovered the same phenomenon in eyes compensating for lenses under dim illumination.⁴⁰ We speculate that this may be a reflection of a process that normally drives the two eyes toward a common refraction (i.e., an isometropizing influence). This “anti-yoking” phenomenon was not found in the rate of ocular elongation, in that the fellow eyes of positive and negative lens-wearing eyes changed by roughly the same amount (Fig. 5B and 5D).

Implications for Human Myopia

The development of myopia in school-age children has long been associated with the presumed hyperopic defocus the eyes experience during reading.^{41,42} One of the puzzles of the etiology of human myopia is that although there is much epidemiological evidence drawing a strong association of reading or other near-work with the incidence of myopia, studies explicitly looking for such evidence within a population by correlating rates of myopic progression with hours spent at near-work have yielded generally weaker associations.^{43–47} Given the distinctively different fall-times of ocular compensation caused by positive and negative lens-wear in chicks, it is likely that the total amount of near work does not capture all the temporal information used for integration of the defocus signals. Our findings that the effect of episodes of lens-wear saturate after a few minutes and that the endurance of the effect of episodes of myopic defocus from wearing positive lenses is greater than that of negative lenses might provide a basis for the hypothesis that it is not the total duration of hyperopic or myopic defocus that is important, but the particular temporal pattern of the defocus.

Indeed, without waiting for scientific verification, there is widespread encouragement of children to take frequent breaks from reading and to view distant objects, particularly in Singapore and China. One can speculate that the existence of a transient myopia after periods of near-work⁴⁸ might mean that such breaks have something in common with the positive lenses used in our study.

The recent growing awareness that ocular elongation is influenced not only by vision at the fovea, but by the entire retina (reviewed by refs. 4 and 49) also gives heightened significance to temporal factors, in that, in daily life, any given retinal region would experience an alternation of myopic and hyperopic defocus as the objects imaged on that region happen to be nearer or further from the focal plane depending on the level of accommodation, which is determined largely by the fovea. Furthermore, even if we ignore these spatial inhomogeneities, whatever it is about reading or other near-work that causes myopic progression will be present in a complex temporal pattern as the child interrupts reading by viewing distant objects. We

view this study as a step towards understanding the temporal properties of emmetropization. Given that a study of this sort could not be carried out on children, we suggest that correlating the pattern of reading and breaks with the rate of progression of myopia may prove useful.

Acknowledgments

Supported by National Institute of Health Grants EY-02727 and RR-03060

References

1. Norton TT, Siegwart JT Jr. Animal models of emmetropization: matching axial length to the focal plane. *J Am Optom Assoc* 1995;66:405–414. [PubMed: 7560727]
2. Edwards MH. Animal models of myopia. A review. *Acta Ophthalmol Scand* 1996;74:213–219. [PubMed: 8828713]
3. Wildsoet CF. Active emmetropization--evidence for its existence and ramifications for clinical practice. *Ophthalmic Physiol Opt* 1997;17:279–290. [PubMed: 9390372]
4. Wallman J, Winawer J. Homeostasis of eye growth and the question of myopia. *Neuron* 2004;43:447–468. [PubMed: 15312645]
5. Kroger RH, Wagner HJ. The eye of the blue acara (*Aequidens pulcher*, Cichlidae) grows to compensate for defocus due to chromatic aberration. *J Comp Physiol [A]* 1996;179:837–842.
6. Schaeffel F, Glasser A, Howland HC. Accommodation, refractive error and eye growth in chickens. *Vision Res* 1988;28:639–657. [PubMed: 3195068]
7. Irving EL, Sivak JG, Callender MG. Refractive plasticity of the developing chick eye. *Ophthalmic Physiol Opt* 1992;12:448–456. [PubMed: 1293533]
8. McFadden SA, Howlett MH, Mertz JR. Retinoic acid signals the direction of ocular elongation in the guinea pig eye. *Vision Res* 2004;44:643–653. [PubMed: 14751549]
9. Siegwart JT Jr, Norton TT. The susceptible period for deprivation-induced myopia in tree shrew. *Vision Res* 1998;38:3505–3515. [PubMed: 9893785]
10. Metlapally S, McBrien NA. The effect of positive lens defocus on ocular growth and emmetropization in the tree shrew. *J Vis* 2008;8:1–12. [PubMed: 18484807]
11. Smith EL 3rd, Maguire GW, Watson JT. Axial lengths and refractive errors in kittens reared with an optically induced anisometropia. *Invest Ophthalmol Vis Sci* 1980;19:1250–1255. [PubMed: 7419376]
12. Whatham AR, Judge SJ. Compensatory changes in eye growth and refraction induced by daily wear of soft contact lenses in young marmosets. *Vision Res* 2001;41:267–273. [PubMed: 11164443]
13. Hung LF, Crawford ML, Smith EL. Spectacle lenses alter eye growth and the refractive status of young monkeys. *Nature Med* 1995;1:761–765. [PubMed: 7585177]
14. Wallman J, Wildsoet C, Xu A, et al. Moving the retina: choroidal modulation of refractive state. *Vision Res* 1995;35:37–50. [PubMed: 7839608]
15. Howlett MH, McFadden SA. Form-deprivation myopia in the guinea pig (*Cavia porcellus*). *Vision Res* 2006;46:267–283. [PubMed: 16139323]
16. Troilo D, Nickla DL, Wildsoet CF. Choroidal thickness changes during altered eye growth and refractive state in a primate. *Invest Ophthalmol Vis Sci* 2000;41:1249–1258. [PubMed: 10798638]
17. Hung LF, Wallman J, Smith EL 3rd. Vision-dependent changes in the choroidal thickness of macaque monkeys. *Invest Ophthalmol Vis Sci* 2000;41:1259–1269. [PubMed: 10798639]
18. Winawer J, Wallman J. Temporal constraints on lens compensation in chicks. *Vision Res* 2002;42:2651–2668. [PubMed: 12445851]
19. Napper GA, Brennan NA, Barrington M, Squires MA, Vessey GA, Vingrys AJ. The effect of an interrupted daily period of normal visual stimulation on form deprivation myopia in chicks. *Vision Res* 1997;37:1557–1564. [PubMed: 9231222]
20. Flitcroft DI. The lens paradigm in experimental myopia: oculomotor, optical and neurophysiological considerations. *Ophthalmic Physiol Opt* 1999;19:103–111. [PubMed: 10615446]
21. Wallman J, Adams JI. Developmental aspects of experimental myopia in chicks: susceptibility, recovery and relation to emmetropization. *Vision Res* 1987;27:1139–1163. [PubMed: 3660666]

22. Nickla DL, Wildsoet C, Wallman J. Visual influences on diurnal rhythms in ocular length and choroidal thickness in chick eyes. *Exp Eye Res* 1998;66:163–181. [PubMed: 9533843]
23. Zhu X, Park TW, Winawer J, Wallman J. In a matter of minutes, the eye can know which way to grow. *Invest Ophthalmol Vis Sci* 2005;46:2238–2241. [PubMed: 15980206]
24. Zhu X, Winawer J, Wallman J. The potency of myopic defocus in lens-compensation. *Investigative Ophthalmology and Visual Science* 2003;44:2818–2827. [PubMed: 12824218]
25. Napper GA, Brennan NA, Barrington M, Squires MA, Vessey GA, Vingrys AJ. The duration of normal visual exposure necessary to prevent form deprivation myopia in chicks. *Vision Res* 1995;35:1337–1344. [PubMed: 7610595]
26. Schmid KL, Wildsoet CF. Effects on the compensatory responses to positive and negative lenses of intermittent lens wear and ciliary nerve section in chicks. *Vision Res* 1996;36:1023–1036. [PubMed: 8736261]
27. Shaikh AW, Siegwart JT Jr, Norton TT. Effect of interrupted lens wear on compensation for a minus lens in tree shrews. *Optom Vis Sci* 1999;76:308–315. [PubMed: 10375247]
28. Smith EL 3rd, Hung LF, Kee CS, Qiao Y. Effects of brief periods of unrestricted vision on the development of form-deprivation myopia in monkeys. *Invest Ophthalmol Vis Sci* 2002;43:291–299. [PubMed: 11818369]
29. Kee CS, Hung LF, Qiao-Grider Y, et al. Temporal constraints on experimental emmetropization in infant monkeys. *Invest Ophthalmol Vis Sci* 2007;48:957–962. [PubMed: 17325132]
30. Winawer J, Zhu X, Choi J, Wallman J. Ocular compensation for alternating myopic and hyperopic defocus. *Vision Res* 2005;45:1667–1677. [PubMed: 15792842]
31. Norton TT, Siegwart JT Jr, Amedo AO. Effectiveness of hyperopic defocus, minimal defocus, or myopic defocus in competition with a myopiagenic stimulus in tree shrew eyes. *Invest Ophthalmol Vis Sci* 2006;47:4687–4699. [PubMed: 17065475]
32. Kee C, Marzani D, Wallman J. Lens-compensation myopia is not the same as form-deprivation myopia. *Invest Ophthalmol Vis Sci*. (ARVO Suppl) 1998;39:S715.
33. Nickla, DL. Ph. D. dissertation. City University of New York; 1996. Diurnal Rhythms and Eye Growth in Chicks.
34. Park T, Winawer J, Wallman J. Further evidence that chicks use the sign of blur in spectacle lens compensation. *Vision Res* 2003;43:1519–1531. [PubMed: 12782066]
35. Nickla DL, Wilken E, Lytle G, Yom S, Mertz J. Inhibiting the transient choroidal thickening response using the nitric oxide synthase inhibitor L-NAME prevents the ameliorative effects of visual experience on ocular growth in two different visual paradigms. *Exp Eye Res* 2006;83:456–464. [PubMed: 16635488]
36. Kusakari T, Sato T, Tokoro T. Visual deprivation stimulates the exchange of the fibrous sclera into the cartilaginous sclera in chicks. *Exp Eye Res* 2001;73:533–546. [PubMed: 11825024]
37. Mertz JR, Wallman J. Choroidal retinoic acid synthesis: a possible mediator between refractive error and compensatory eye growth. *Exp Eye Res* 2000;70:519–527. [PubMed: 10866000]
38. Feldkaemper MP, Wang HY, Schaeffel F. Changes in retinal and choroidal gene expression during development of refractive errors in chicks. *Invest Ophthalmol Vis Sci* 2000;41:1623–1628. [PubMed: 10845578]
39. Wildsoet C, Wallman J. Choroidal and scleral mechanisms of compensation for spectacle lenses in chicks. *Vision Res* 1995;35:1175–1194. [PubMed: 7610579]
40. Rucker FJ, Wallman J. Cone signals for spectacle lens compensation: Differential responses to short and long wavelengths. *Vision Res*. 2008in press.
41. Gwiazda J, Thorn F, Bauer J, Held R. Myopic children show insufficient accommodative response to blur. *Invest Ophthalmol Vis Sci* 1993;34:690–694. [PubMed: 8449687]
42. Rosenfield, M.; Gilmartin, B. Myopia and Nearwork: causation or merely association?. In: Rosenfield, M.; Gilmartin, B., editors. *Myopia and nearwork*. Oxford: Butterworth-Heinemann; 1998. p. 193-206.
43. Saw SM, Katz J, Schein OD, Chew SJ, Chan TK. Epidemiology of myopia. *Epidemiol Rev* 1996;18:175–187. [PubMed: 9021311]

44. Tan NW, Saw SM, Lam DS, Cheng HM, Rajan U, Chew SJ. Temporal variations in myopia progression in Singaporean children within an academic year. *Optom Vis Sci* 2000;77:465–472. [PubMed: 11014673]
45. Saw SM, Wu HM, Seet B, et al. Academic achievement, close up work parameters, and myopia in Singapore military conscripts. *Br J Ophthalmol* 2001;85:855–860. [PubMed: 11423462]
46. Saw SM, Chua WH, Hong CY, et al. Nearwork in early-onset myopia. *Invest Ophthalmol Vis Sci* 2002;43:332–339. [PubMed: 11818374]
47. Mutti DO, Mitchell GL, Moeschberger ML, Jones LA, Zadnik K. Parental myopia, near work, school achievement, and children's refractive error. *Invest Ophthalmol Vis Sci* 2002;43:3633–3640. [PubMed: 12454029]
48. Ciuffreda KJ, Ordonez X. Abnormal transient myopia in symptomatic individuals after sustained nearwork. *Optom Vis Sci* 1995;72:506–510. [PubMed: 8539015]
49. Stone RA, Flitcroft DI. Ocular shape and myopia. *Ann Acad Med Singapore* 2004;33:7–15. [PubMed: 15008555]

FIGURE 1.

Schematics showing the linear model (**A**) and the nonlinear model (**B**) of the hypothesized internal emmetropization signal. (**A**) The linear model has the magnitude of the signal being proportional to the sign and duration of defocus experienced over time, and the signal stays the same when no defocus is present. (**B**) The nonlinear model has the signal rising and becoming saturated during defocus, and it declines slowly between episodes of defocus.

FIGURE 2.

Schematics showing the rationale for determining the rise-time (A) and the fall-time (B) of the hypothesized emmetropization signals. (A) Giving repeated lens-wearing episodes with different durations at a fixed frequency allows us to estimate how fast the signal rises. (B) Giving saturating lens-wearing episodes of a fixed duration but at various intervals, allows us to estimate how fast the signal declines.

FIGURE 3.

The rise-times of the hypothesized internal emmetropization signals for positive and negative lens-wear for ocular length (A) and choroidal thickness (B). Values plotted are Relative Changes ($\Delta X - \Delta N$). See Table 1 for the statistical significance of the values. Continuous positive and negative lens-wear is plotted at 60 and 10 min, respectively. Overlapping data points were shifted slightly left or right for clarity. The dashed curve in (A) is fit to all data; the solid curve omits an outlying group in which birds wore lenses for 2 min every 10 min. Data points marked with $\dagger$ or $\S$ differ significantly from those marked with $\dagger\dagger$ or $\S\S$, respectively, by ANOVA with LSD *post hoc* tests. Error bars, SEM.

FIGURE 4.

The fall-times of the hypothesized emmetropization signals for both positive and negative lens-wear for ocular length (A) and choroidal thickness (B). Values plotted are Relative Changes ($\Delta X - \Delta N$). See Table 1 for the statistical significance of the values. Continuous spectacle lens-wear is plotted at 0.01 hr. Overlapping data points were shifted slightly left or right for clarity. The negative lens-wear data points fell into 3 clusters that were significantly different from one another by ANOVA with LSD *post hoc* tests and were marked with $\dagger$, $\dagger\dagger$, or $\dagger\dagger\dagger$; the positive lens-wear data points marked with $\S$ significantly differ from those marked with $\S\S$ by ANOVA with LSD *post hoc* tests. Error bars, SEM. The horizontal line indicating 95% CI for ocular length is obscured by the adjacent data point.

FIGURE 5.

The effects of darkness and yoking on fellow eyes: (A) the mean changes in choroidal thickness of the fellow eyes vs. the amount of total darkness per day, (B) the mean changes in ocular length of the fellow eyes vs. the amount of total darkness per day, (C) the mean changes in choroidal thickness of the fellow eyes vs. those of the treated eyes, and (D) the mean changes in ocular length of the fellow eyes vs. those of the treated eyes, for Exp. 2. Long periods of daily darkness caused progressively more choroidal thinning (A), but had little consistent effect on the rate of ocular elongation (B) in the fellow eyes (continuous lens-wear is shown at 10 hr of darkness for eyes wearing positive (filled diamond) or negative (open diamond) lenses and their fellow eyes; third order polynomial fit to the means from each group). Even though the choroidal changes in the fellow eyes are positively correlated with those in the treated eyes (C), the choroids of the fellow eyes of birds wearing negative lenses became thicker on average than those of birds wearing positive lenses (A and C); this “anti-yoking” was not observed for the rate of ocular elongation (B and D). Squares are averages; unfilled circles are birds wearing negative lenses; filled circles are birds wearing positive lenses. Error bars, SEM.

—●— fellow eyes of eyes with periods of darkness (present study)
 —○— fellow eyes of eyes without periods of darkness (ref. 24)

FIGURE 6.

Changes in choroidal thickness of the fellow eyes as a function of the initial choroidal thickness. The correlation was greater in eyes with multiple periods of darkness each day than in eyes in normal light in between episodes of lens-wear.

Table 1
Summary of Treatment Protocols and Measured Changes in Experimental Eyes (AX) and Fellow Eyes (AN, Mean ± SEM)

Exp.	Treatment Paradigm	+7 D Lenses										-7 D Lenses										
		Interval					Ocular Length (μm)					Choroidal Thickness (μm)					Vitreous Chamber Depth (μm)					
		Group	n	AX	AN	ΔX	AX	AN	ΔX	AX	AN	ΔX	AX	AN	ΔX	AX	AN	ΔX				
Exp. 1 Rise-Time	10 sec	I	9	202 ± 27	218 ± 24	-35 ± 15	-45 ± 17	111 ± 35	155 ± 26	-1.1 ± 0.6	-0.8 ± 0.5	10 min	9	10	223 ± 24	185 ± 16	-48 ± 19	-50 ± 15	129 ± 21	104 ± 16	-4.5 ± 0.4	-1.5 ± 0.6
	30 sec	2	10	176 ± 21	253 ± 26	-46 ± 14	-44 ± 8	103 ± 25	183 ± 25	-1.0 ± 0.4	-1.7 ± 0.3											
	1 min	3	15	154 ± 24	222 ± 23	-3 ± 14	-27 ± 14	43 ± 19	134 ± 29	0.4 ± 0.5	-0.5 ± 0.5	10 min	10	10	292 ± 24	235 ± 24	-29 ± 18	-16 ± 25	216 ± 28	121 ± 30	-3.5 ± 0.7	-0.7 ± 0.7
	2 min	4	11	99 ± 48	281 ± 30	-16 ± 15	-42 ± 16	-22 ± 36	192 ± 28	0.5 ± 0.6	-0.2 ± 0.9											
	2 min	5	7	-12 ± 42	199 ± 27	5 ± 19	-33 ± 19	-40 ± 50	192 ± 19	4.2 ± 0.6	0.6 ± 0.4	10 min	11	15	252 ± 34	259 ± 25	-17 ± 10	10 ± 14	161 ± 28	115 ± 19	-1.7 ± 0.6	0.3 ± 0.6
	5 min	6	9	52 ± 21	250 ± 21	28 ± 18	-29 ± 29	-73 ± 31	163 ± 16	4.0 ± 0.8	-0.4 ± 0.4	10 min	12	10	318 ± 23	190 ± 25	-53 ± 17	-12 ± 13	261 ± 20	66 ± 22	-5.8 ± 0.5	-1.2 ± 0.6
	10 min	7	7	54 ± 20	207 ± 24	104 ± 35	-28 ± 16	-162 ± 37	118 ± 27	6.3 ± 0.5	0.9 ± 0.4											
	continuous	8	13	-19 ± 31	176 ± 28	89 ± 17	-10 ± 16	-160 ± 30	65 ± 22	5.5 ± 0.7	-0.8 ± 0.5		13	14	312 ± 37	187 ± 21	-58 ± 26	-3 ± 14	276 ± 16	61 ± 19	-3.6 ± 0.5	-0.2 ± 0.4
	Exp. 2 Fall-Time	Dark Interval																				
		0.5 hr	14	6	10 ± 40	242 ± 43	81 ± 15	10 ± 24	-192 ± 23	63 ± 32		NA		20	8	348 ± 32	301 ± 19	-18 ± 12	43 ± 22	263 ± 33	99 ± 19	-3.8 ± 0.5
1.5 hr		15	23	33 ± 19	243 ± 18	101 ± 16	-5 ± 11	-159 ± 29	116 ± 19	6.2 ± 0.6	-0.4 ± 0.3		21	18	256 ± 22	260 ± 18	-45 ± 15	31 ± 13	235 ± 23	99 ± 20	-3.3 ± 0.4	-0.3 ± 0.3
3.5 hr		16	7	103 ± 26	279 ± 13	58 ± 26	-26 ± 17	-56 ± 36	189 ± 17	2.7 ± 0.7	-1.8 ± 0.5		22	17	290 ± 27	289 ± 17	-26 ± 10	16 ± 13	217 ± 23	133 ± 24	-3.0 ± 0.5	0.0 ± 0.3
5.5 hr		17	7	89 ± 46	293 ± 34	-8 ± 16	-4 ± 12	12 ± 36	184 ± 22	1.6 ± 0.7	-1.3 ± 0.4		23	24	217 ± 21	227 ± 17	-71 ± 13	-30 ± 11	182 ± 16	142 ± 13	-4.6 ± 0.8	-1.3 ± 0.4
11.5 hr		18	21	137 ± 17	276 ± 21	-18 ± 13	-37 ± 9	49 ± 22	199 ± 16	1.2 ± 0.5	-1.0 ± 0.4		24	10	253 ± 28	269 ± 23	-39 ± 14	-5 ± 14	153 ± 25	112 ± 20	-1.9 ± 0.6	0.1 ± 0.3
23.5 hr		19	8	100 ± 18	163 ± 28	-33 ± 8	-62 ± 11	22 ± 17	116 ± 21	2.0 ± 0.6	-0.4 ± 0.5		25	7	160 ± 38	172 ± 39	-46 ± 19	-23 ± 14	109 ± 40	101 ± 34	-2.0 ± 0.6	-0.1 ± 0.5
47.5 hr																						

Data shown are changes in the treated and fellow eyes over the course of 3-day experiments. The changes that were significantly different from each other by paired, 2-tailed Student *t*-tests are shown in bold and underlined. In Experiment 1, episodes were repeated every hr or every 10 min. In Experiment 2, episodes lasted 30 min and were repeated with frequencies from hourly to every other day.